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# Digesta Passage Time, Digestibility, and Total Gut Fill in Captive Japanese Macaques (*Macaca fuscata*): Effects Food Type and Food Intake Level

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1 Digesta passage time, digestibility and total gut fill in captive Japanese

2 macaques: the effects of food type and food intake level

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19    **Abstract**

20            Digestion is an important process in understanding the feeding ecology  
21    of animals. We examined digesta passage time, digestibility, and total gut fill in  
22    Japanese macaques ( $n = 4$ ) under four diet conditions representing the seasonal  
23    and regional variations in the diets of wild populations to determine the effects of  
24    food type and food intake on these digestive features. Food type was  
25    associated with mean retention time (MRT), digestibility, and total gut fill. Dry  
26    matter intake (DMI) of food was positively correlated with total gut fill but not with  
27    MRT or digestibility. Indigestible DMI, on the other hand, affected MRT  
28    negatively. Thus, when Japanese macaques consume high-fiber foods, MRT  
29    becomes shorter and digestibility is lower than eating low-fiber foods.  
30    Moreover, macaques experience increases in total gut fill when they consume  
31    high-fiber diets or a large amount of food. Japanese macaques may excrete  
32    difficult-to-digest food components quickly: they nevertheless buffer an increase  
33    in food intake by an increase in gut fill. Our study offers new insight into the  
34    relationship between feeding ecology and nutritional physiology in primates by  
35    simultaneously examining the effects of food type and intake level on MRT and  
36    digestibility.

37

38 Key words: digesta passage time; mean retention time; digestibility; total gut

39 fill; *Macaca fuscata*

40



## 41 Introduction

42

43           Animals must absorb nutrients and energy from the foods they ingest  
44 and digest (Robbins 1983). Since the amount of food they need to eat highly  
45 depends on how efficiently they can digest food, digestibility must be taken into  
46 account to understand energy balance. Plant foods are rich in hard-to-digest  
47 structural carbohydrates such as cellulose and hemicellulose. The relatively  
48 large and/or sometimes compartmentalized gastrointestinal tracts and symbiotic  
49 gut microbes of herbivores enable them to retain such fibrous foods for a longer  
50 time period and to carry out adequate bacterial fermentation. In herbivores,  
51 digestion is a time-dependent process (Clauss *et al.* 2007), and the longer the  
52 ingesta is retained in the tract, the better digestibility becomes (Stevens and  
53 Hume 1998). Mean retention time (MRT) is the most reliable single measure to  
54 evaluate the digesta passage time. Digestibility becomes higher with an  
55 increase in MRT values in ungulates (Illius and Gordon 1992; Clauss *et al.* 2007).  
56 A recent review by Clauss *et al.* (2008) also found the same positive relationship  
57 between MRT and apparent digestibility of neutral detergent fiber (NDF; largely  
58 consisting of hemicellulose, cellulose and lignin) among primates, such as

59 lemurs (Edwards and Ullrey 1999a; Campbell *et al.* 2004), howler monkeys, and  
60 colobus monkeys (Edwards and Ullrey 1999b).

61         There are two types of digestive systems in primates; caeco-colic and  
62 forestomach fermentations (Lambert 1998). Most primate species, including  
63 Japanese macaques, are caeco-colic fermenters with a relatively large colon  
64 and/or enlarged caecum for extended microbial fermentation. The colobines,  
65 like nonruminant herbivorous species such as hippos, tree kangaroos and sloths,  
66 on the other hand, are forestomach fermenters having an enlarged, sacculated  
67 forestomach, which enables the animals to carry out extended microbial  
68 fermentation (Chivers 1994; Chivers and Langer 1994; Stevens and Hume 1998).  
69 In general, caeco-colic fermenting animals feed on foods with relatively  
70 digestible components, whereas forestomach fermenting animals rely on foods  
71 with high-fiber contents (Lambert 1998).

72         Wild primates feed on various kinds of foods, reflecting seasonal and  
73 regional differences. Japanese macaques in different regions adopt different  
74 feeding strategies, and diet composition, activity budget, and home range size  
75 are affected by the differences in food availability (Nakagawa 1997; Hanya 2004;  
76 Tsuji and Takatsuki 2004). For example, Japanese macaques in both

77 warm-temperate evergreen forests and cool-temperate deciduous forests  
78 confront difficulties in meeting caloric and nutritional requirements during winter  
79 (Nakagawa *et al.* 1996). In the evergreen forest of Koshima Island, for example,  
80 the major food resource for macaques during winter is mature leaves. These  
81 leaves contain high levels of fiber and low levels of easily digestible  
82 carbohydrates (Iwamoto 1982), but nevertheless food intake is high enough to  
83 compensate for the low diet quality. In the deciduous forest of Kinkazan Island,  
84 on the other hand, macaques feed mainly on winter buds and tree barks, which  
85 also have a relatively low nutritional quality. These food items are so small that  
86 the macaques fail to increase food intake enough to offset the low diet quality.  
87 Based on observations of feeding behavior and nutritional analysis of their foods,  
88 Nakagawa (1989) revealed that energy intake of Japanese macaques during  
89 winter was not enough to cover maintenance energy requirements. In addition  
90 to consumption of fat accumulated during autumn (Wada *et al.* 1975), Japanese  
91 macaques may have physiological adaptations that enable them to meet  
92 nutritional and caloric needs when they confront such bad food conditions. If  
93 macaques are capable of increasing retention time to prolong time for bacterial  
94 fermentation under a food shortage, for example, they might need less food and

thus could save time and energy searching for foods. Alternatively, they might be able to improve digestive activity by increasing total gut fill in response to changes in food conditions, as in some rodent species (El-Harith *et al.* 1976; Owl and Batzli 1998; Naya *et al.* 2005).

In this study, we simulated variable food environments that wild populations would face using captive Japanese macaques to determine the effects of food type and food intake level on mean retention time (MRT), digestibility and total gut fill as an index of gut intake capacity. We predict that MRT becomes longer in response to an increase in indigestible material intake level to have enough time for fiber digestion and prevent a decrease in digestibility. We also examined the prediction that total gut fill increases when the macaques consume more indigestible materials as reported in rodents. This study offers new insight into the relationship between feeding ecology and nutritional physiology in primates by simultaneously examining the effects of food type and intake level on MRT and digestibility.

## Materials and methods

## Study Subjects

We conducted this study on four individually housed adult male Japanese macaques (mean 13.6 kg body weight; 10.5 years old) at the Research Resource Station of the Primate Research Institute, Kyoto University (Table I). All of the animals were born and raised in captivity. Their usual diets consist of artificial pellets with moderate-level fibers, along with some fruits and vegetables such as apples and sweet potatoes. We also give them a piece of wood so that they can nibble the tree bark. The animals did not have any of these additional food items during each experiment. We carried out the experiments from January 21, 2008 to March 29, 2008.

## Experimental Diets

To investigate the effect of NDF content of food on MRT and digestibility, we used high-fiber (NDF 37.5%; Oriental Yeast Co., Ltd.) and low-fiber diets (NDF 13.6%, PMI Nutrition International) (Table II). During the first experiment, we gave the animals a small amount (166 g/day in dry matter (DM), or 180 g/day

as fed) of high-fiber pellets to study the effects on MRT and digestibility (Table III).

In the next experiment, we fed them a large amount (230 g DM, 250 g as-fed) of

the same high-fiber pellets. In the following two experiments, we gave the

animals low-fiber pellets in the same amounts (Small: 169 g DM, 180 g as-fed;

Large: 235 g DM, 250 g as-fed). Thus, we set four feeding conditions:

High-Small, High-Large, Low-Small and Low-Large. Low-Large represents the

situation where high-quality food is abundant, whereas High-Small reflects the

worst case scenario that macaques in deciduous forests would face during

winter. Daily dry matter intake (DMI) of High-Small was 166 g and the daily

energy intake was 531 kcal (physiological fuel value; provided by the

manufacturer).

## Feeding Trials

Each time before we started feeding the animals a new type of food, we

set a 3-day introduction period and a 5-day adaptation period. The first 3 days

were to gradually change their diets from the original to the experimental ones.

During the next 5 days, the animals consumed only experimental diets so that

we could exclude possible effects of the original diets they had previously had, although gut microbes may take more than 8 days to adapt to the experimental diet. We fed the animals twice a day, at 10:00h and at 15:00h, and quantified the amount of food at the individual level. The animals consumed all of the food given and water was available ad libitum. We checked body weight of the animals before and after each experiment to maintain their good health (Table IV).

#### Measurement of Digesta Passage

We used chromium mordanted onto cell-wall constituents (Cr-CWC; 0.08 g/BW kg) prepared from alfalfa (*Medicago sativa*) as the particle Cr marker and Cobalt-ethylene diaminetetraacetic acid (Co-EDTA; 0.04 g/BW kg) as the fluid Co marker (Udén *et al.* 1980; Caton *et al.* 1999). We mixed the marker dose into a piece of pancake and gave it to the animals at 8:00 on Day 1 of each trial before their morning meals.

We set a wire-mesh sheet on four legs under each cage so that we could easily separate feces from urine. After marker dosing at 8:00h, we monitored

the animals every 2 hours for the first 12 hours, then every 4 hours for the next 12 hours (Day 1 - Day 2). We observed the animals every 6 hours for the next 24 hours (Day 2 – Day 3) and every 8 hours for the last 72 hours (Day 3 – Day 6) (Sakaguchi *et al.* 1991). After collecting samples, we deep-froze them immediately at -30 °C, and then vacuum-dried them at 60 °C until reaching a constant weight. Next, we ground dried samples and stored them in plastic tubes. For determination of chromium (Cr) and cobalt (Co), we ashed each fecal sample at 550 °C for 6 hours in a muffle furnace. Then we dissolved the ashed samples in 0.1 N hydrochloric acid solution. We determined Cr and Co concentration in the treated sample by atomic absorption spectroscopy (Atomic absorption spectrophotometer AA-660, Shimadzu, Kyoto).

#### Measurement of Digestibility

We determined apparent digestibility of DM (aD DM) and NDF (aD NDF) in each trial. To carry out nutritional analysis for each feeding trial, we pooled all the feces of the last 96 hours, from 8:00h on Day 2 to 8:00h on Day 6, for each animal (Table III). We did not use feces collected prior to this period as an



precaution in addition to the 5-day adaptation period since the marker analysis suggested that complete marker excretion may take over 100 hours.

To estimate aD DM, we also needed to determine food intake during the same period. Since we finished the experiment before AM meal on Day 6, we considered the total food intake of each trial as the sum of food given from Day 2 to Day 5. We determined aD NDF in duplicate from 0.5 g samples using the methods of Van Soest *et al.* (1991). After removing crude fat from samples by the Soxhlet method with a diethyl-ether extract, we boiled them in an NDF solution for 1 hour. We dried the NDF residues and then calculated NDF by subtracting the ashed residues from them.

## Data Analysis

We calculated the particle Cr and fluid Co MRT of each animal according to the formula of Blaxter *et al.* (1956):

$$MRT(h) = \frac{\sum_{i=1}^N M_i T_i}{\sum_{i=1}^N M_i},$$

where  $M_i$  is the amount of the marker excreted in the  $i$ th defecation and  $n$  is the

total number of defecations. We regarded  $T_i$  as the middle of the sampling interval so that the calculated MRT would become a better indication of the true MRT without frequent sampling (Van Weyenberg *et al.* 2006).

We used the following formula for apparent digestibility (Robbins 1983):

$$aD (\%) = \frac{\text{Amount consumed} - \text{Fecal excretion}}{\text{Amount Consumed}} \times 100$$

,based on an average daily food intake and excretion over 4 days.

We estimated the amount of Indigestible DMI ( $\text{g/kg}^{0.75}/\text{d}$ ) using the following formula:

$$\text{Indigestible DMI} = \text{DMI} - (\text{DMI} \cdot A)$$

where  $A$  is the fractional digestibility of the diet.

We calculated total gut fill of the animals as follows (Holleman and White 1989):

$$V = V_N + \frac{V_N \cdot A}{2(1 - A)}$$

where  $V_N$  is the indigestible material fill. Of the two formulae provided by

Holleman and White (1989), we chose the equation based on the assumption

that absorption of ingested food would occur linearly with time. We did not take the fluid Co marker MRT into account since the marker used in this calculation must be representative of solid ingesta (Holleman and White 1989).

We analyzed MRT values and digestibility using a generalized linear model (GLM), where food type and intake level were independent variables (food type: High = 0, Low = 1; food intake level: Large = 0, Small = 1), in R for Windows version 2.9.2 (2009 The R Foundation for Statistical Computing). We selected the function with the minimum Akaike Information Criterion (AIC) as the best-fitted model for each feeding trial. We also analyzed the relationship between MRT and digestibility as well as that between indigestible DMI and MRT.

## Results

### Digesta Passage

MRT of both the particle Cr and fluid Co markers became shorter under high-fiber diets (Fig. 1). The best-fit model selected for the particle Cr included

only food type (Cr MRT =  $12.425 \times \text{food type} + 35.125$ , AIC = 110.37, df = 15, F = 13.553,  $P = 0.002$ ). Although the best-fit model for the fluid Co included both food type and food intake level (Co MRT =  $16.038 \times \text{food type} + 5.613 \times \text{food intake level} + 26.456$ , AIC = 114.77, df = 15, F = 10.131,  $P = 0.002$ ), a model including only food type also had a small AIC (Co MRT =  $16.038 \times \text{food type} + 29.262$ , AIC = 115.28, df = 15, F = 16.614,  $P = 0.001$ ), suggesting that food type had a stronger effect on the MRT of the fluid Co markers. The differences in MRT for the two diet types were 12 hours for the particle Cr marker (high-fiber:  $35.1 \pm 1.9$  h, low-fiber:  $47.5 \pm 9.3$  h; mean  $\pm$  SD) and 16 hours in the fluid Co marker (high-fiber:  $29.3 \pm 3.3$  h, low-fiber:  $45.3 \pm 10.6$  h). The amount of food intake level had no effect on MRT of the both markers.

The amount of indigestible material in the four diets was largest in High-Large, then High-Small, Low-Large, and Low-Small (Fig. 2, Table IV). MRT of both markers became shorter with increasing indigestible DMI (Pearson's correlation, Cr:  $r = -0.748$ , df = 14,  $P < 0.001$ , Co:  $r = -0.819$ , df = 14,  $P < 0.001$ , Fig. 3), although this relation was not very clear in the high-fiber diets, where the animals consumed a relatively large amount of indigestible materials.

## 257 Digestibility

258 Both aD DM and aD NDF measured in the high-fiber diets were lower  
259 than those of low-fiber diets. The best-fit model selected for aD DM included  
260 only food type (aD DM =  $26.297 \times \text{food type} + 56.897$ , AIC = 72.50, df = 15, F =  
261 640.73,  $P < 0.0001$ ). Both food type and food intake level showed effects on  
262 aD NDF (aD NDF =  $11.469 \times \text{food type} - 4.601 \times \text{food intake level} + 54.746$ , AIC  
263 = 100.38, df = 15, F = 13.199,  $P < 0.001$ ), although food type appeared to be  
264 more influential than food intake level (aD NDF =  $11.469 \times \text{food type} + 54.746$ ,  
265 AIC = 102.34, df = 15, F = 19.234,  $P < 0.001$ ; aD NDF =  $-4.601 \times \text{food intake level}$   
266 + 60.48, AIC = 114.56, df = 15, F = 1.4065,  $P = 0.255$ ). High-fiber diets had  
267 more than 25% lower aD DM compared to low-fiber diets ( $56.9 \pm 1.9\%$  and  $83.2$   
268  $\pm 2.3\%$ , mean  $\pm$  SD, respectively), and aD NDF of high-fiber diets was also lower  
269 than that of low-fiber diets ( $53.6 \pm 4.4\%$  and  $62.9 \pm 9.0\%$ , respectively; Table IV).

270 MRT and aD DM were correlated with each other in the trials with  
271 low-fiber diets, but not in those with high-fiber diets (Fig. 4). Since food intake  
272 did not have an influence on MRT values, we pooled all data from the four trials  
273 and then divided them into two groups based on food type. As a result, we  
274 found a significant correlation between the particle Cr MRT and aD DM in the

low-fiber diet trials (Pearson's correlation, Cr:  $r = 0.722$ ,  $df = 6$ ,  $P = 0.043$ ),  
although the slope of the regression was not steep. There was no such  
significant correlation between the fluid Co MRT and aD DM (Co:  $r = 0.695$ ,  $df =$   
 $6$ ,  $P = 0.056$ ). MRT was not correlated with either aD DM of high-fiber diets  
(Pearson's correlation, Cr:  $r = -0.124$ ,  $df = 6$ ,  $P = 0.769$ , Co:  $r = 0.432$ ,  $df = 6$ ,  $P =$   
 $0.286$ ) or aD NDF of both high-fiber (Cr:  $r = 0.285$ ,  $df = 6$ ,  $P = 0.494$ , Co:  $r =$   
 $0.385$ ,  $df = 6$ ,  $P = 0.346$ ) and low-fiber (Cr:  $r = -0.036$ ,  $df = 6$ ,  $P = 0.933$ , Co:  $r = -$   
 $0.084$ ,  $df = 6$ ,  $P = 0.843$ ) diets.

283

284 Total Gut Fill

285

Total gut fill was associated with both food type and intake level. The  
best-fit model for total gut fill included both food type and intake level (Total gut  
fill =  $-67.309 \times \text{food type} - 28.031 \times \text{food intake level} + 142.044$ , AIC = 128.30,  $df$   
= 15,  $F = 80.111$ ,  $P < 0.0001$ ). Total gut fill became greater when the animal fed  
the diets with either larger amounts or higher fiber levels (Fig. 5).

291

292 **Discussion**

293

294           Two limitations of our study may influence our results. First, we used  
295   NDF rather than acid detergent fiber (ADF) as a parameter of fiber content.  
296   ADF permits more precise comparisons because NDF contains partially  
297   digestible hemicellulose. When two diets given to gorillas contained similar  
298   levels of NDF, the one with high ADF content showed a low digestibility (Remis  
299   and Dierenfeld 2004). However, the high-fiber pellets are also likely to be high  
300   in ADF due to the large difference in NDF levels of the two diets we used (37.5%  
301   in high-fiber; 13.6% in low-fiber, respectively). Second, we had no information  
302   on NDF composition. Hemicellulose is more digestible and lignin is less  
303   digestible, so the higher aD NDF of the low-fiber diet might suggest a high  
304   hemicellulose content, whereas the high-fiber diet contains more lignin. Such  
305   differences in NDF composition may affect aD DM of the two diets. However,  
306   even if that was the case, our result would not be very different because of  
307   distinct differences in both the NDF content and aD DM of the two diets.

308

309   Effects of Food Type

310

311           This study shows that MRT measured in both particle and fluid markers  
312   become much shorter when the Japanese macaques feed in the high-fiber diets.  
313   This tendency has been reported for other primate species including langurs  
314   (Nijboer *et al.* 2007) and chimpanzees (Milton and Demment 1988), and is likely  
315   to e because high-fiber diets contain more indigestible materials, which push the  
316   digesta out to the gut, and thus shorten the MRT.

317           Both the apparent digestibility of dry matter and NDF (aD DM, aD NDF,  
318   respectively) depended on food type. A higher aD DM in the low-fiber diets is  
319   consistent with the previous studies on lemurs (Edwards and Ullrey 1999a),  
320   gorillas (Remis and Dierenfeld 2004), orangutans (Schmidt *et al.* 2009), and  
321   howler and colobus monkeys (Edwards and Ullrey 1999b), and the same  
322   negative correlation exists between aD NDF and fiber contents of diets (Schmidt  
323   *et al.* 2009). Since high-fiber foods contain more indigestible material that  
324   inhibits absorption of nutrients, it is reasonable to find a lower aD DM and aD  
325   NDF.

326           Food type also affects total gut fill of Japanese macaques. Greater  
327   total gut fill in the High-Small diet compared to the Low-Large diet implies a more  
328   significant effect of food type than food intake. The differences in total gut fill



among the four diets may indicate the feeding strategy of Japanese macaques in different food environments. When food contains a lot of fiber, the macaques can meet their energy requirement either by increasing food intake level or decreasing MRT down to a point where digestibility might be compromised (Fig. 3). Once their MRT bottoms out due to high indigestible DMI, the macaques can react by increasing total gut fill in order to prevent further drops in MRT.

#### Effect of Food Intake Level

DMI had little effect on both MRT and digestibility in Japanese macaques. This result differs from previous studies on herbivores, where MRT became shorter as DMI increased (Fryxell *et al.* 1994; Clauss *et al.* 2004; Clauss *et al.* 2007), and primates, where a review of studies on 19 species found a significant negative correlation between MRT and DMI (Clauss *et al.* 2008). This difference in results may be due to a difference in the range of food intake levels, which is expressed as relative DMI ( $\text{g/kg}^{0.75}/\text{d}$ ). Relative DMI in this study is 10.2 - 21.0  $\text{g/kg}^{0.75}/\text{d}$  (Table IV), which is smaller than the 11 - 118  $\text{g/kg}^{0.75}/\text{d}$  range of the previous study (Clauss *et al.* 2008). However, the food intake level

of wild Japanese macaques usually does not range that wide, and the relatively small range in food intake level (190 - 299 DM g/d over a 6-month period) among macaques in the evergreen forest of Koshima (Iwamoto 1982) suggests that the food intake level in our experiment was reasonable.

Indigestible DMI affected the particle Cr marker MRT while food intake level had little effect on MRT. The more the animals ingested indigestible materials, the shorter the particle Cr MRT became, although this tendency was very weak in high-fiber diets since MRT becomes more or less constant once indigestible DMI reaches a certain level ( $5 \text{ g/kg}^{0.75}/\text{d}$ , approximately) (Fig. 3). The variation in the particle Cr MRT among the four diets (High-Large, High-Small, Low-Large, and Low-Small) does not seem to be as great as that found in indigestible DMI, since there is little difference in MRT between High-Large and High-Small diets (Fig. 1). This may indicate that the amount of indigestible material in High-Small is high enough to bring MRT close to the minimum level, where additional indigestible material in High-Large would no longer affect MRT.

DMI was associated with total gut fill of Japanese macaques. High-Large diet showed greater total gut intake than High-Small diet, and

Low-Large diet showed greater total gut intake than Low-Small diet (Fig. 5). Japanese macaques may be capable of increasing DMI by increasing total gut fill when they need to consume a large amount of food. Based on the combined effects of both food type and intake level on total gut fill, we conclude that Japanese macaques have a flexible digestive tract that enables them to deal with different food conditions.

## The Digestive Strategy of Japanese Macaques

The results of this study indicate that aD DM measured in the low-fiber diets become higher when MRT is longer. However, the correlation was small since the range of aD DM (79.4 – 85.6%) was much smaller than that of MRT (Cr: 34.9 – 60.2 h; Co: 31.6 – 59.9 h). Thus, we could not firmly conclude that aD DM was improved by increased MRT. Compared to low-fiber diets, high-fiber diets showed a much smaller range of MRT (Cr: 32.6 – 38.6 h; Co: 24.6 – 34.2 h), so we could not examine the influence of MRT on aD DM in the high-fiber diets.

A longer MRT is one way to deal with a high-fiber diet because microbial

383 fermentation of structural carbohydrates requires time. This is true for  
384 leaf-eating primates such as the colobines (Edwards and Ullrey 1999b; Nijboer  
385 *et al.* 2007), but not for Japanese macaque since they have a shorter MRT and  
386 lower aD DM in the high-fiber diets. Our results indicate that the macaques  
387 have a flexible digestive system that allows them to deal with various food  
388 conditions and that there seems to be a gut capacity threshold around 5 g  
389 indigestible DMI/kg<sup>0.75</sup>/d. Below this threshold, the more indigestible materials  
390 the macaques ingest, the shorter MRT becomes. Once they reach the  
391 threshold by consuming high-fiber foods or a relatively large amount of low-fiber  
392 foods, their gut simply expands. These findings suggest that Japanese  
393 macaques ensure MRT never becomes too short; not less than 30 h for particles.  
394 Some rodent species are also known to have a flexible digestive strategy to  
395 meet their energy requirements, increasing the size of the digestive tract in  
396 response to temperature (Naya *et al.* 2005) or diet types (El-Harith *et al.* 1976;  
397 Owl and Batzli 1998; Naya *et al.* 2005), suggesting better digestion due to  
398 increased fermentative activity.  
399  
400 Implications for the Feeding Ecology of Wild Japanese Macaques

401

402           Our results indicate that Japanese macaques use different food  
403   processing strategies in different food environments. When low-fiber food is  
404   available and indigestible DMI range is low, macaques excrete difficult-to-digest  
405   foods quickly so that they can increase their food intake level. When food  
406   contains a lot of fiber, they can meet their energy requirement either by  
407   increasing food intake level or developing a greater total gut fill.

408           During winter, macaques in evergreen forests can live on mature leaves,  
409   which are low in energy content (Iwamoto 1982). However, since leaves are  
410   large in size, they can stuff themselves with such food in a short feeding time.  
411   In such a food environment, the macaques may meet their energy requirements  
412   by increasing food intake level and/or by developing a greater gut fill. In winter  
413   deciduous forests, on the other hand, few mature leaves are available and  
414   macaques eat mainly winter buds and tree barks, which reduce intake rate and  
415   are difficult to digest (Nakagawa 1989). These animals must therefore survive  
416   even severer food conditions. Macaques in deciduous forests lose their body  
417   weight in winter by consuming fat deposited during autumn (Wada 1975; Wada  
418   *et al.* 1975; Koganezawa 1995). One possible way to deal with such a situation

419 is to increase gut capacity so that they can retain indigestible materials in the gut  
420 long enough to maintain fiber digestibility. Having an almost constant particle  
421 MRT in the high indigestible DMI range may imply such an ability. The fact that  
422 the fecal microflora of wild Japanese macaques in a snowy district was different  
423 from that of captive ones (Benno *et al.* 1987) might also imply that wild Japanese  
424 macaques have another digestive function due to unique intestinal microflora  
425 Thus, our finding might not fully explain feeding adaptations in wild Japanese  
426 macaques and further study of wild macaques is required.

427

428 In conclusion, we found that the digestibility of high-fiber food is lower  
429 than that of low-fiber food regardless of food intake level. Our findings suggest  
430 that Japanese macaques are capable of dealing with various food conditions by  
431 adopting different food processing strategies, under the strong influence of  
432 indigestible DMI level. Macaques excrete difficult-to-digest materials quickly in  
433 the low indigestible DMI range, while they have a constant MRT once  
434 indigestible DMI exceeds a threshold. These results demonstrate the need to  
435 take food type or indigestible DMI into account when comparing MRT and  
436 digestibility within or among primate species.

437

438

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440

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## Figure legends

Fig. 1 Mean retention time (MRT; h) of two markers by Japanese macaques among four feeding conditions. Box indicates upper and lower quartiles; horizontal line indicates the median; whiskers indicate the range.

Fig. 2 Indigestible DMI in four feeding conditions.

Fig. 3 The relationship between indigestible DMI and particle Cr MRT.

Fig. 4 Relationships between MRT of the markers and apparent digestibility.

(a) aD DM (b) aD NDF with the particle Cr marker MRT, (c) aD DM and (d) aD NDF with the fluid Co marker MRT.

Fig. 5 Total gut fill in four feeding conditions.

Table I. Details of the animals used in this study

Species	Animal	Born	Age (year)	Origin	Sex	BW (kg)
<i>M. fuscata</i>	1	1995	13	Captive	Male	13.2
<i>M. fuscata</i>	2	1995	13	Captive	Male	16.4
<i>M. fuscata</i>	3	1997	11	Captive	Male	14.7
<i>M. fuscata</i>	4	1999	9	Captive	Male	11.8

BW (body weight) was measured on January 20<sup>th</sup>, 2008, a day prior to the start of the first experiment.

Table II. Major nutritional values of two commercial pellets and energy intake of four feeding conditions

	High-Fiber *	Low-Fiber **
Crude protein (%)	18.6	25.9
Crude fat (%)	3.4	4.7
NDF (%)	37.5 <sup>a</sup>	13.6
Crude ash (%)	12.1	5.7
Physiological fuel value (kcal / g)	2.95	3.40
kcal / Large	737.5	850.0
kcal / Small	531.0	612.0

All values are expressed on a fresh basis.

NDF (neutral detergent fiber) = hemicellulose, cellulose and lignin

Physiological fuel value (kcal / g) = Sum of decimal fractions of proteins, fat and carbohydrate x 4, 9, 4 kcal / g, respectively

\* Diet for Zoo Animal (ZF), Oriental Yeast Co. Ltd, Tokyo

\*\* Certified Primate Diet, PMI Nutrition International, Montana

<sup>a</sup> Data from Sakaguchi *et al.* (1999)

Table III. Mean  $\pm$  SD food intake and feces excretion for each feeding trial

Diet	Food intake		Feces excretion	
	As fed (g)	DM (g)	DM (g)	Frequency
High-Large	1002.8 $\pm$ 2.3	921.5 $\pm$ 2.2	400.2 $\pm$ 14.6	14.0 $\pm$ 0.0
High-Small	723.3 $\pm$ 0.1	664.7 $\pm$ 0.1	284.2 $\pm$ 15.5	11.8 $\pm$ 1.5
Low-Large	1004.7 $\pm$ 0.6	940.0 $\pm$ 0.6	155.4 $\pm$ 20.7	10.3 $\pm$ 1.5
Low-Small	722.9 $\pm$ 0.2	676.3 $\pm$ 0.2	117.7 $\pm$ 16.1	9.0 $\pm$ 1.4

605 Food intake is expressed both in fresh (As fed) and dry matter (DM).  
606 Frequency indicates the number of times that we collected fecal samples out of  
607 14 collection times. All values are measured over 96 hours excluding first 24  
608 hours.  
609 High-Large = high-fiber diet in a large amount; High-Small = high-fiber diet in a  
610 small amount; Low-Large = low-fiber diet in large amount; Low-Small = low-fiber  
611 diet in small amount

612 Table IV. Food and indigestible intake, MRT, digestibility and body weight of the animals under four feeding conditions

Diet	Animal	BW		DMI		indigestible DMI		MRT		aD	
		Initial (kg)	Final (kg)	(g/day)	(g/kg <sup>0.75</sup> /day)	(g/day)	(g/kg <sup>0.75</sup> /day)	Cr (h)	Co (h)	DM (%)	NDF (%)
High-Large	1	13.2	13.0	230.1	33.2	96.0	13.9	35.3	31.9	58.3	48.2
	2	16.4	16.4	230.3	28.3	99.0	12.2	34.4	26.3	57.0	55.6
	3	14.7	13.5	230.2	30.7	104.8	14.0	37.1	28.4	54.5	52.7
	4	11.8	10.7	231.2	36.3	100.4	15.8	35.2	24.6	56.6	55.1
High-Small	1	13.0	12.6	166.1	24.3	70.5	10.3	34.2	32.5	57.5	48.9
	2	16.3	15.9	166.1	20.5	69.7	8.6	38.6	34.2	58.0	55.3
	3	14.2	13.7	166.1	22.7	67.4	9.2	32.6	28.8	59.4	53.6
	4	11.6	11.6	166.1	26.4	76.6	12.2	33.6	27.4	53.9	50.1
Low-Large	1	12.8	12.5	235.3	34.8	33.9	5.0	49.9	51.2	85.6	64.9
	2	16.3	16.2	235.2	29.0	38.9	4.8	42.7	41.3	83.4	71.9
	3	14.0	13.7	235.0	32.5	36.5	5.0	48.8	40.5	84.4	68.7
	4	11.2	10.6	234.8	38.4	46.0	7.5	34.9	31.6	80.4	66.6
Low-Small	1	13.0	12.9	169.1	24.7	25.2	3.7	56.0	55.2	85.1	52.3
	2	16.4	16.6	169.1	20.7	26.3	3.2	53.0	51.0	84.5	66.9
	3	13.5	14.1	169.1	24.0	29.3	4.2	60.2	59.9	82.7	65.2
	4	10.7	11.1	169.0	28.6	34.8	5.9	34.9	31.7	79.4	54.9

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617 Diet: High = high-fiber diet, Low = low-fiber diet, Large = large amount, Small =  
618 small amount; Initial BW = body weight of the animals measured before each  
619 feeding trial; Final BW = body weight measured after each trial; DMI = dry matter  
620 intake; MRT = mean retention time of the two markers (Cr: particle, Co: fluid); aD  
621 DM = apparent DM digestibility; aD NDF = apparent NDF digestibility